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Ultra-low fouling and functionalizable zwitterionic coatings grafted onto SiO_2 via a biomimetic adhesive group for sensing and detection in complex media

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ABSTRACT

Non-specific protein binding from human plasma and serum has severely hindered the full capabilities of biosensors concerned with cancer biomarker detection. Currently, there is a strong desire for developing new materials which allow for the convenient attachment of an ultra-low fouling and functionalizable surface coating which can be used for highly sensitive and label-free detection of target analytes directly from complex media. In this work, a short 20 min *in situ* "graft to" protocol using Tris pH 8.5 buffer was developed for zwitterionic carboxybetaine methacrylate (CBMA) polymer conjugates containing the adhesive biomimetic moiety, 3,4-dihydroxy-L-phenylalanine (DOPA), on SiO_2 substrates. Using a surface plasmon resonance (SPR) biosensor, different buffers, pH values, salt concentrations, and temperatures were investigated for determining the "graft to" conditions that yield dense polymer films which both minimize non-specific protein adsorption and maximize antibody immobilization. The optimized surface coatings were shown to be highly protein resistant to 100% human blood plasma and serum. Subsequent antibody functionalized surfaces without any blocking agents enabled the specific detection of the cancer biomarker ALCAM directly from undiluted human serum down to 64 ng/mL. The successful use of this zwitterionic surface coating for detection from complex media on SiO_2 surfaces indicates its potential for broad impacts in the development of implantable medical devices, *in vivo* diagnostics, and nano-scale biosensors.

1. Introduction

The inability to identify and verify suitable biomarkers using various sensing devices has been the major shortcoming in cancer diagnostics (Benson et al., 2006; Hanash et al., 2008; Sawyers, 2008). As many of these low concentration proteins are found in complex bodily fluids (e.g. plasma, serum, urine) (Feng et al., 2009; Radpour et al., 2009) the culprit underlying detection using biosensors is the overwhelming background noise as a result of non-specific protein adsorption (Cheng et al., 2006; Ferrari, 2005; Paulovich et al., 2008; Ullah and Aatif, 2009).

Recent studies have shown the ability of polymers containing the carboxybetaine (CB) moiety, formed via surface initiated atom transfer radical polymerization (SI-ATRP), to outperform commonly used protein resistant ethylene glycol (EG) based coatings. These polyCB (pCB) films were shown to be ultra-low fouling to 100% human plasma and serum (Ladd et al., 2008; Vaisocherova et al., 2008; Yang et al., 2009). It has been hypothesized that the surface hydration formed via ionic solvation due to the zwitterionic groups is primarily responsible for the ability of CB polymers to prevent protein adsorption (Chen et al., 2005). Subsequent functionalization with antibodies using the abundant carboxylic acid groups and convenient *N*-hydroxysuccinimide (NHS)/*N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide (EDC) chemistry enabled sensitive and specific detection of analytes directly from undiluted human plasma (Vaisocherova et al., 2008).

However, many biosensors today incorporate the use of a variety of nanomaterials with differing geometries into their design (Chen et al., 2004; Zhang et al., 2009). The dimensions of the sensing surface have also been decreasing to the nanometer length scale (Kim et al., 2009). Therefore, other methods for creating a bifunctional coating on such devices, capable of highly sensitive detection from complex media, need to be developed.

As an alternative to SI-ATRP, there has been much attention focusing on developing "graft to" technologies due to their simplicity and convenience (Ryu et al., 2005). However, this technique requires a method for chemically attaching the non-fouling material "to" the substrate via some form of an adhesive moiety. During a study of surface modification strategies which could be applied

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to a variety of materials (Lee et al., 2007) it was found that the compound 3,4-dihydroxy-L-phenylalanine (DOPA) could strongly adhere to numerous substrates (noble metals, native oxides, ceramics, etc.).

Several studies on the ability of PEG polymers containing DOPA anchors for forming protein resistant coatings on oxide surfaces have been performed (Dalsin et al., 2005). It was found that the number of residues in the DOPA anchor significantly affected the surface density of the grafted polymer and its subsequent ability to resist protein adsorption. In previous work (Li et al., 2008), a single DOPA anchor conjugated to polymers composed of zwitterionic sulfobetaine methacrylate (SBMA) was shown to form protein resistant coatings on various surfaces. To increase the surface density of the grafted zwitterionic polymers and provide for functionalization capabilities, two linked DOPA–poly(carboxybetaine methacrylate) ($DOPA_2$–$pCBMA_2$) polymer conjugates was synthesized and grafted to gold substrates (Gao et al., 2010). Following antibody immobilization, the detection of a target protein directly from undiluted human plasma was demonstrated using a SPR biosensor.

Conventional SPR sensor chips are composed of a top layer of gold (~48 nm) for which many surface chemistries have been developed. However, many other materials are incorporated into the design of biosensors today which include silica, titanium, platinum, among others (Archakov and Ivanov, 2007; Chen et al., 2004; Homola, 2008; Zhang et al., 2009). Thus, there has been a push to use available sensing platforms as tools for developing new surface chemistries for these materials. Silica is an attractive material for many biosensor platforms due to the widely established use of silicon (from which SiO_2 is formed) in the semiconductor industry which has enabled the development of convenient micro-fabrication methods. Silicon is currently used in many microelectromechanical system (MEMS) devices (Zhu et al., 2005) and has since been applied to developing nanoelectromechanical system (NEMS) based biosensors with recent advances in nanotechnology (Burg et al., 2007). Hence, new surface chemistries for this material need to be developed.

In this work, the "graft to" behavior of novel $DOPA_2$–$pCBMA_2$ polymer conjugates onto silica substrates was studied using a SPR sensor. The grafting conditions were investigated in order to both minimize biofouling and maximize protein immobilization efficiency. The ability of the $DOPA_2$–$pCBMA_2$ surface functionalized with antibodies to detect analytes directly from undiluted human blood serum was demonstrated using the cancer biomarker, activated leukocyte cell adhesion molecule (ALCAM, CD 166). Using a simple 20 min *in situ* attachment protocol, the detection of ALCAM at concentrations down to ~64 ng/mL was enabled.

2. Materials and methods

2.1. Materials

$DOPA_2$–$pCBMA_2$ polymer conjugates were synthesized as described previously (Gao et al., 2010). Sodium hydroxide (99.998%), tetrahydrofuran (THF, HPLC grade), *N*-hydroxysuccinimide (NHS), and *N*-ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were purchased from Sigma–Aldrich (Milwaukee, WI). Tris, crystallized free base (molecular biology grade) was purchased from Fisher Scientific. Fibrinogen (fraction I from bovine plasma), lysozyme (from chicken egg white), and 3-(*N*-morpholino)propanesulfonic acid (MOPS, minimum 99.5%), and phosphate-buffered saline (PBS, 0.01 M phosphate, 0.138 M sodium chloride, 0.0027 M potassium chloride, pH 7.4) were purchased from Sigma Chemical Company. Sodium chloride and sodium phosphate were purchased from J.T. Baker (Phillipsburg, NJ). Anhydrous sodium acetate was purchased from Fluka BioChemika. Pooled human plasma and serum were purchased from BioChemed Services (Winchester, VA). Human monoclonal antibodies against the activated leukocyte cell adhesion molecule (anti-ALCAM) and human recombinant ALCAM/Fc chimera were purchased from R&D Systems (Minneapolis, MN). Rabbit polyclonal biotinylated antibody to Salmonella sp. was purchased from Meridian Life Science Inc. (Saco, MA). Ethanol (200 proof) was purchased from Decon Laboratories. The water used was purified using a Millipore water purification system with a minimum resistivity of 18.0 MΩ cm.

2.2. SPR sensor and substrates

A laboratory SPR sensor developed at the Institute of Photonics and Electronics, Prague, Czech Republic was used (Homola, 2008) as described previously (Vaisocherova et al., 2008). This custom built SPR is based on the attenuated total reflection method and wavelength modulation. It is equipped with a four-channel flow cell, temperature control, and uses a peristaltic pump for delivering samples.

SPR sensor chips were made of a glass slide coated with an adhesion-promoting titanium film (~2 nm), a gold film (~48 nm), and an additional titanium film (~1 nm), for promoting the adhesion of the SiO_2 film, by an electron beam evaporator. The substrates were then immediately coated with ~20 nm of SiO_2 by plasma enhanced chemical vapor deposition (PECVD). Prior to being mounted onto the SPR sensor, the chips were rinsed with ethanol, placed in a UV ozone cleaner for 20 min, rinsed with Millipore water and ethanol, and then blown dry with filtered air.

2.3. Calibration of the SPR sensor response

The sensitivity of a SPR sensor depends on the distance of the binding event from the SPR active surface (Homola, 2008). Since an additional 1 nm titanium film and ~20 nm SiO_2 film were coated on top of the regular SPR chips, the sensitivity had to be calibrated. The sensitivity calibration factor was determined experimentally and found to be 1.40 (i.e. a 1 nm wavelength shift on the silica substrates was equivalent to a 1.4 nm shift on the standard SPR substrates). For more information regarding the experimental calibration see Supporting Information.

A theoretical model for the silica coated SPR substrate was also developed to predict the change in surface sensitivity. The model revealed a calibration factor of 1.33, which was in good agreement with the experimental measurement (within 5.3%). The difference is mostly caused by the accuracy of the dielectric constants used in the theoretical calculation. Therefore, a calibration factor of 1.37, an average of the experimental and theoretic results, was used for this study. Hence, for the SPR sensor used with the silica coated SPR chips, a 1 nm shift in resonant wavelength corresponded to a change in protein surface coverage of ~23 ng/cm^2 (Homola, 2006).

2.4. Grafting $DOPA_2$–$pCBMA_2$ to the substrates

$DOPA_2$–$CBMA_2$ polymer was grafted to the silica substrate *in situ* by flowing the polymer solution over the sensor chip using Tris buffer (2.5 mg/mL) for 20 min at a flow rate of 40 μL/min. After injecting the polymer solution, the surface was then rinsed with water followed by PBS before quantifying the net surface coverage.

2.5. Surface functionalization

The functionalization procedure was monitored step by step in real-time using the SPR sensor at a flow rate of 40 μL/min and 25 °C.

Following polymer attachment, the carboxylate groups were activated by injecting a solution consisting of 0.1 M NHS and 0.4 M EDC in water for 7 min. After briefly flowing 10 mM sodium acetate buffer (SA, pH 5.0), a solution of antibody dissolved in 10 mM Tris–HCl (pH 9.0, 50 μg/mL) was flowed for 11 min. A buffer solution containing 10 mM sodium phosphate and 0.3 M NaCl (PBNa, pH 8.2) was subsequently injected for 10 min to wash the surface followed by SA buffer to reestablish the baseline for quantifying the antibody surface coverage.

2.6. Measurements of non-specific protein adsorption

The non-specific protein adsorption for the polymer conjugate attached to the silica substrate was determined by flowing PBS buffer and then protein solutions consisting of 1 mg/mL of fibrinogen and lysozyme in PBS, 100% human plasma, and 100% human serum in four separate flow channels followed by PBS.

2.7. Measurements of specific protein binding

ALCAM was selected to demonstrate the specific protein biomarker detection abilities of the antibody functionalized $DOPA_2$–$pCBMA_2$ surfaces in both buffer and undiluted human serum. For the detection in buffer, both anti-ALCAM and reference anti-Salmonella were immobilized using the above protocol. After establishing a stable baseline with PBS buffer, PBS spiked with ACLAM at increasing concentrations (26, 64, 160, 398, and 1000 ng/mL) was then subsequently injected for 10 min followed by rinsing with buffer for 10 min between samples. The net specific (or non-specific in the case of anti-Salm.) protein binding for each concentration was taken as the difference between the initial and incremental PBS baselines. For the detection in undiluted human serum, only anti-ALCAM was immobilized on all four channels using the above protocol. After establishing a stable baseline with PBS, samples of ALCAM spiked 100% human serum at six concentrations (26, 64, 160, 280, 398, and 1000 ng/mL) were then flowed through three channels and unspiked serum was flowed through the fourth channel followed by PBS for an additional 15 min.

3. Results and discussion

3.1. SiO_2 coated SPR chips

In order for the SiO_2 thin film coated onto the SPR chips to serve as a model system for developing new surface chemistries for biosensing applications, it must be compatible to a variety of conditions (i.e. various temperatures, pHs, solvents, etc.) and maintain its stability during detections. Prior to grafting $DOPA_2$–$pCBMA_2$ onto the substrates, the stability of the SiO_2 layer was tested under a variety of pH values (1–10) and solutions/organic solvents (e.g. 3 M NaCl in PBS, DMF/water, THF, ethanol, and acetone). It was found that the SiO_2 layers formed on conventional SPR chips mediated with 1 nm of titanium were very stable. However, when the film was formed directly on the gold surface of the chip, it was washed away during experiments (data not shown). Furthermore, the change in SPR surface sensitivity due to both the titanium interlayer and SiO_2 film was found to be minor from both experimental measurements and theoretical calculations. Little variation in the characteristics of the SPR wavelength dips was also observed. Therefore, substrates composed of 20 nm of SiO_2 and 1 nm of titanium on conventional SPR chips were used for the remainder of the study.

Several attempts for making SiO_2 coated SPR chips have been reported previously. Some used a thin titanium interlayer between the silica and gold films to promote adhesion (Glasmastar et al., 2002; Graneli et al., 2003) but some did not (Szunerits et al., 2006).

Table 1
$DOPA_2$–$pCBMA_2$ polymer surface coverage and total non-specific protein adsorption from 100% human blood plasma under different "graft to" conditions.[a].

Condition	Polymer surface coverage [ng/cm^2]	Non-specific protein adsorption (100% human plasma) [ng/cm^2]
pH 3	212.9	10.5
pH 6	217.1	11.4
pH 7	223.8	6.5
pH 8	273.7	5.6
pH 8.5	283.2	5.4
pH 9	262.2	6.1
pH 10	265.0	6.8
pH 8.5, 40 °C	271.8	6.4
pH 8.5 + 10 mM NaCl	282.7	5.1
pH 8.5 + 1 M NaCl	212.4	7.9

[a] Polymer surface coverage and non-specific protein adsorption using 10 mM Tris–HCl. The polymer was first dissolved in buffer at 2.5 mg/mL and then flowed over the sensor chip for 20 min at a flow rate of 40 μL/min. Unless otherwise specified, each condition was run at 25 °C. For protein fouling experiments, following polymer attachment, a stable PBS baseline was obtained. Undiluted human plasma was then flowed over the sensor surface for 10 min followed by rinsing with buffer for 10 min.

The SiO_2 thin film deposited directly on a gold surface via PECVD was demonstrated to be stable in both acidic and basic solutions as well as organic solvents (Szunerits et al., 2006). However, our results showed that the SiO_2 thin film formed via this method was very unstable just after a few days and was easily removed by water or organic solvents alike. While PECVD is a common method for the formation of silica films, little variation in the experimental parameters can cause significant differences in the quality of the layer formed (e.g. silanol and water impurities, porosity) which may explain the different resulting film characteristics (Adams et al., 1981; Ceiler et al., 1995).

3.2. $DOPA_2$–$pCBMA_2$ grafted to SiO_2 substrates and non-specific protein adsorption

As shown in Fig. 1a, the first step for obtaining sensitive and specific detection is to achieve a high surface coverage of pCBMA polymer which can minimize non-specific protein adsorption. In order to maximize the surface density of the non-fouling and functionalizable CB groups on silica substrates using the "graft to" method, the $DOPA_2$–$pCBMA_2$ structure shown in Fig. 1b was used.

Previous investigations of the adhesive mechanism for DOPA have been conducted (Lee et al., 2006). It was found that under non-oxidizing conditions (e.g. neutral pH) a strong metal–oxygen coordination complex can form between catecholic DOPA and an oxide surface thereby creating an adhesive interaction with high dissociation energy. As oxidation of the side chains occur, DOPA cross-linking reactions begin to take place. This was found to be an important mechanism for forming dense films using DOPA polymer conjugates (Gao et al., 2010). Therefore, a variety of grafting conditions were studied in order to optimize the surface coverage for the silica substrates.

Tris buffer was selected due to its ability to allow a strong attachment of DOPA onto a variety of surfaces (Lee et al., 2006). The polymer surface coverage under different pH values, salt concentrations, and temperatures for a fixed flow time of 20 min and the protein fouling properties of these films are summarized in Table 1. The conditions of high salt (~1 M) and temperature (40 °C) were tested because it was reported that $DOPA_3$-PEG conjugates were able to form dense polymers on TiO_2 substrates in such an environment (Dalsin et al., 2005). Here, the results indicated that the amount of bound polymer was largest in the range of pH values of 8–10, decreased with decreasing pH and high ionic

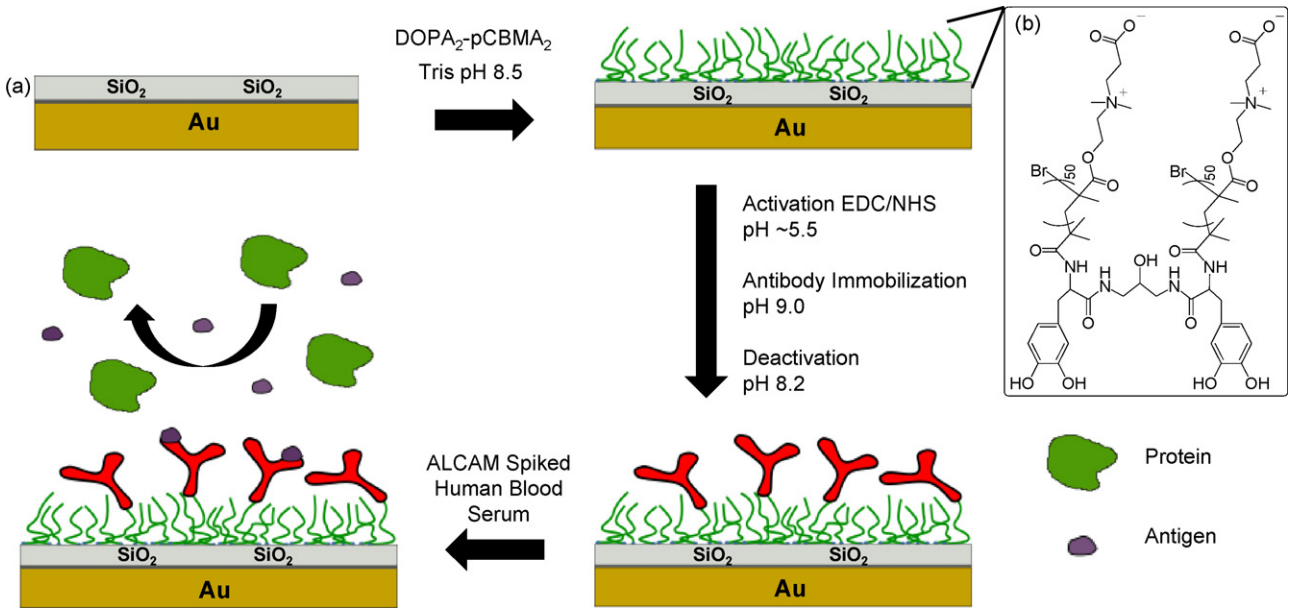

Fig. 1. (a) The newly developed protocol for a novel "graft to" method which allows for highly sensitive detection from undiluted human serum on SiO_2 using a SPR biosensor. Starting with a cleaned silica substrate, the $DOPA_2$–$pCBMA_2$ polymer conjugate is grafted to the sensing surface. Carboxylic groups on the polymer are activated using EDC/NHS chemistry which can then react with primary amines on antibodies. Following deactivation of the residual activated groups, the functionalized surface is used for specific detection of a target analyte from human serum. The low fouling background combined with good immobilization efficiency allows for highly sensitive detection. (b) The $DOPA_2$–$pCBMA_2$ polymer conjugate. The two DOPA anchors combined with the two bifunctional CBMA polymer chains allow for a high surface coverage of both non-fouling and functionalizable groups on silica substrates.

strengths, and was not affected by temperature over the range tested (as limited by the SPR equipment). The optimal "graft to" condition was found to be 10 mM Tris buffer pH 8.5 at 25 °C, in which the $DOPA_2$–$pCBMA_2$ surface coverage was found to be 260.1 ± 18.1 ng/cm^2. As shown in Supporting Information Fig. S1, very little polymer was removed during the washing steps indicating a strong and mostly permanent attachment onto the SiO_2 surface. The adsorption of 1 mg/mL fibrinogen and lysozyme solutions for the optimized polymer surface was undetectable (data not shown). The protein fouling to undiluted human plasma and serum were 6.3 ± 0.9 and 11.7 ± 3.0 ng/cm^2, respectively. The sensor-gram for a typical fouling experiment using undiluted human plasma and serum is shown in Fig. 2.

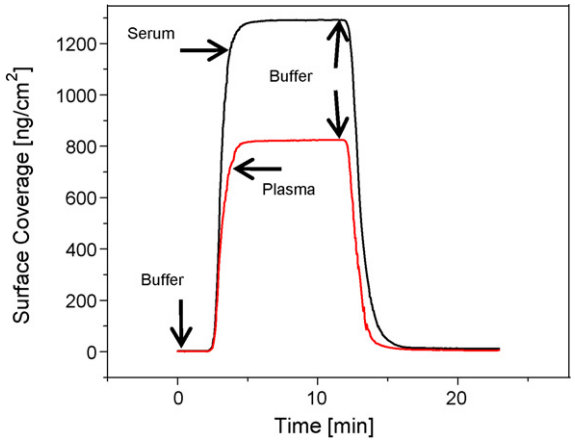

Fig. 2. SPR response to flowing undiluted human plasma and serum over the $DOPA_2$–$pCBMA_2$ coated surface. After obtaining a stable baseline using buffer, each protein solution was flowed for 10 min at 40 μL/min followed by rinsing with PBS for an additional 10 min. The sensor response for determining the non-fouling properties of the surface coating was calculated as the difference between the starting and ending buffer baselines. Here, the total biofouling to human plasma and serum was 5.4 and 9.5 ng/cm^2, respectively.

The observation that the polymer surface coverage was decreased under low pH conditions (pH ≤7) could be attributed to the ionizable carboxylate groups present in the pCBMA chains (pK_a ~3.5) (Wielema and Engberts, 1990) and the working range of Tris buffer (pH 7–9.2). When the pH of the original Tris solution (without polymer) approaches the lower limit of the working range, there is minimal buffering capacity. Upon dissolution of $DOPA_2$–$pCBMA_2$, the pH of the solution drops due to the release of protons from the ionizable carboxylate groups. As this occurs, the decreasing pH causes the polymer to take on a slight positive charge as a result of approaching the pK_a of the CB groups. Additionally, the lower pH also creates a slight positive charge on the silica surface (Bousse et al., 1991). The resulting effect is charge–charge repulsion which reduces the overall polymer surface coverage. However, for all pH values tested, low fouling to 100% human plasma was obtained indicating that despite the decrease in coverage, enough of the polymer conjugate was still adsorbed in order to make the surface protein resistant. Since the attachment of the polymer in Tris pH 8–10 buffer at 25 °C was shown to offer the best surface coverage and subsequent non-fouling properties, a pH of 8.5 was used for all further experiments.

As shown in Table 1, high ionic strength also reduced the polymer surface coverage. Water soluble amphoteric polymers are known to exhibit the anti-electrolyte effect at their isoelectric point (IEP). This means that the polymer will unfold from a compact formation upon the addition of salts. However, for uniformly charged polyelectrolytes, when the polymer is present in acidic or basic conditions relative to its IEP, the addition of salt causes the formation of a more compact structure to form, which is more pronounced at higher ionic strengths (Ibraeva et al., 2004). The formation of this more compact structure may have reduced the ability of $DOPA_2$–$pCBMA_2$ to adsorb to the silica surface by forcing the slightly hydrophobic DOPA residues inside the hydrophilic polymer chains thereby decreasing the ability to react with the substrate and anchor the polymer.

Other "graft to" solutions were also tested in an effort to increase the polymer surface coverage. These solutions include water, PBS

(pH 7.4), and MOPS cloud point buffer (pH 6, 3 M NaCl). However, none of the above media resulted in any noticeable improvement in terms of surface coverage or non-fouling.

Recently, our group also studied the "graft to" behavior of $DOPA_2$–$pCBMA_2$ onto gold substrates. It was found that dense polymer films could be formed under cloud point conditions in a THF/pH 3 water mixture (Gao et al., 2010). The resulting surface was shown to be highly protein resistant. This condition was also tested for grafting to silica substrates. While this solution also resulted in low fouling properties using the 20 min attachment protocol (6.3 ng/cm^2 protein adsorption for undiluted blood plasma) the polymer surface coverage was lowered (233.6 ng/cm^2). This indicates that the attachment mechanism for gold and silica substrates could be different. Catechol groups are capable of four different energetic reactions: hydrogen bonding, metal–ligand complexes, Michael-type additions, and charge-transfer complexes (Waite, 1987). Considering the chemical composition and molecular structure of the two substrates, different binding mechanisms would be expected thus requiring different grafting conditions.

3.3. Antibody immobilization

In order to obtain highly sensitive detection, as shown in Fig. 1a, the grafted polymer coating must be able to efficiently immobilize molecular recognition elements. Following polymer attachment, the $DOPA_2$–$pCBMA_2$ coated surface was subsequently immobilized with an antibody for the detection of the cancer biomarker, activated leukocyte cell adhesion molecule (ALCAM, CD 166). ALCAM is a 105 kDa protein with a normal presence in human blood serum (~84 ng/mL). Up-regulation of this protein in the range of ≥100 ng/mL has been shown to be indicative of various cancer types (Faca et al., 2008) indicating the importance for the detection of small changes in concentration.

It was previously determined that a buffer solution with a pH value above the IEP of the antibody was necessary for efficient protein immobilization on pCB surfaces (Vaisocherova et al., 2009). This is due to the slight positive charge on the polymer which is created during activation. Therefore, several different solutions were tested as efficient buffers for this antibody immobilization step. These include sodium carbonate (pK_a 10.25), boric acid (pK_a 9.14), sodium tetraborate decahydrate (pK_a 9.14), and Tris (pK_a 8.08). Sodium hydroxide (pH 10) was previously shown to work well for anti-ALCAM immobilization (Gao et al., 2010; Yang et al., 2009) and was also tested. Further optimization in terms of pH (8.5–10) and buffer concentration (10–100 mM Tris) revealed that 10 mM Tris pH 9.0 offered the best immobilization condition and subsequent activity for anti-ALCAM on the $DOPA_2$–$pCBMA_2$ coated surface. Using similar EDC/NHS activation conditions from previous work (Yang et al., 2009), the anti-ALCAM immobilization levels were 55.4 ± 20.0 ng/cm^2. The results for the four other buffers tested were all less than 20 ng/cm^2. A typical sensor-gram for the antibody immobilization step can be found in Supporting Information Fig. S2.

The previous antibody immobilized levels for pCB films made via SI-ATRP and for $DOPA_2$–$pCBMA_2$ coated gold substrates were found to be 220–330 and 100–130 ng/cm^2, respectively (Gao et al., 2010; Vaisocherova et al., 2008; Yang et al., 2009). The primary reason for the difference amongst the three surfaces could arise from the amount of available pCB groups as a result of both the different surface densities and thicknesses of the polymers. Based on this comparison, the amount of pCB groups available has the following order: pCB by SI-ATRP, $DOPA_2$–$pCBMA_2$ on gold, and $DOPA_2$–$pCBMA_2$ on silica, and thus the immobilization levels would likely follow the same trend.

The antibody to Salmonella (anti-Salm.) was tested to determine the effect of immobilization of different proteins under identical conditions. The immobilization amount of anti-Salm. was found to be ~116 ng/cm^2. This indicates that in cases where an effective monolayer of antibody is not obtained, different antibodies will likely have different immobilization amounts which can likely be attributed to the different IEPs of the proteins. This property results in varying degrees of attraction between the antibody and the activated pCB surface and thus can significantly affect the total amount functionalized.

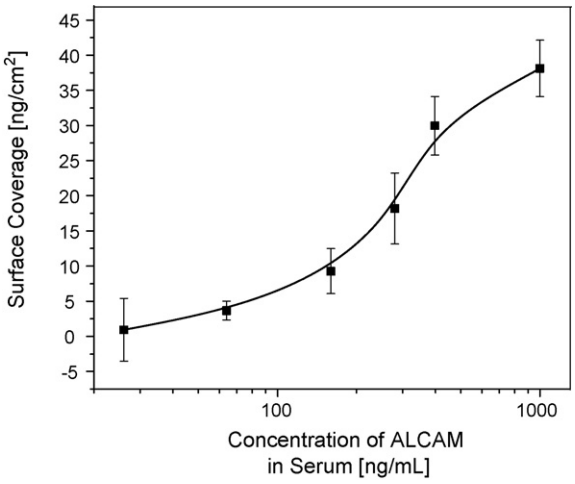

Fig. 3. The detection curve of ALCAM from undiluted human serum. After attachment of the polymer and subsequent functionalization with anti-ALCAM on all four SPR channels (at levels of 55.4 ± 20.0 ng/cm^2) a PBS baseline was obtained. Serum spiked with ALCAM at known concentrations (26–1000 ng/mL) and unspiked serum was flowed through the sensor. The buffer baseline was then reestablished. The net response for each data point was completed in triplicate using *de novo* functionalized surfaces and calculated by subtracting the reference response (i.e. unspiked serum) from each measurement. The detection limit of the unblocked $DOPA_2$–$pCBMA_2$ surface was found to be ~64 ng/mL.

3.4. Detection of target analytes from buffer

The ability of the immobilized antibody to specifically detect low concentrations of target protein was first tested using buffer. Fig. S3 in the Supporting Information shows the cumulative detection curve for ALCAM spiked into PBS at concentrations from 26 ng/mL to 1.0 μg/mL (the raw sensor response converted to protein surface coverage is shown in Supporting Information Fig. S4). The results indicate that the level of anti-ALCAM immobilization was sufficient enough to detect ALCAM in PBS at concentrations down to 26 ng/mL. In order to verify the target specificity of the functionalized surface, the same experiment was performed using the reference antibody, anti-Salm. The results for the highest concentration tested (1 μg/mL) shown in Supporting Information Fig. S5 indicate that despite a higher surface coverage of anti-Salm., there was no non-specific binding of ALCAM, while there was a large specific response on the anti-ALCAM functionalized surface. This indicates that the immobilized anti-ALCAM can specifically bind to the target analyte from buffer while preventing non-specific adsorption.

3.5. Detection of target analytes directly from undiluted human serum

After grafting the polymer to the silica surface and subsequent protein immobilization, the last step, as shown in Fig. 1a, is to detect target analytes from complex media. The resulting detection curve from 100% human serum is shown in Fig. 3. The unblocked $DOPA_2$–$pCBMA_2$ functionalized surface was shown to detect ALCAM directly from undiluted human serum down to con-

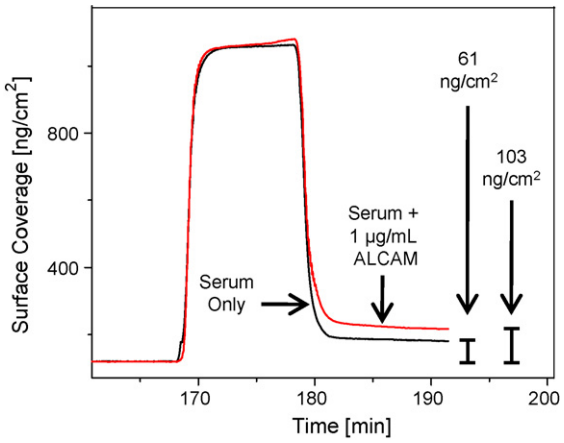

Fig. 4. Specific detection of ALCAM directly from undiluted human serum. After obtaining a stable baseline using buffer, both spiked and unspiked human sera were flowed for 10 min at 40 μL/min followed by rinsing with PBS for an additional 15 min. The lower curve represents the typical response for flowing unspiked serum over the anti-ALCAM functionalized surface. The net response for this curve is due to both specific (ALCAM) and non-specific protein adsorptions. The upper curve represents the response for flowing serum spiked with 1 μg/mL ALCAM, which causes additional specific protein binding of the target antigen. Both channels were functionalized with ~80 ng/cm^2 of anti-ALCAM.

centrations as low as 64 ng/mL. This offers an improvement over the thoroughly blocked OEG COOH/OH alkane thiol-based surface which had a ALCAM limit of detection of >100 ng/mL from human plasma (Vaisocherova et al., 2008). A comparison of the detection curve obtained from serum to that obtained from buffer reveals, that for the same concentration of ALCAM, a larger specific response to the antigen is observed in the buffer solution. This is likely the result of two factors. First, the presence of numerous other proteins in the serum may interfere with the ability of the antigen to reach the surface where it can be detected. Secondly, the presence of non-specific binding on the functionalized surface may block some of the binding sites on the antibody which would further limit the response. In buffer solution, both of these effects do not exist.

Typical sensor responses for the direct detection of ALCAM from undiluted serum are shown in Fig. 4. The difference between the two curves was caused by the additional specific binding of ALCAM in the spiked channel. This shows that the anti-ALCAM functionalized surface can recognize and bind to the target analyte with high specificity even from undiluted human serum. The typical background signal (i.e. the response of the anti-ALCAM functionalized surface to serum only) was found to be ~55 ng/cm^2.

We have previously shown pCB coatings made via SI-ATRP functionalized with an effective monolayer of antibody to sensitively detect spiked ALCAM directly from human plasma at concentrations down to ~10 ng/mL. Furthermore, the DOPA$_2$–pCBMA$_2$ polymer grafted to gold could detect spiked ALCAM down to ~30 ng/mL. Therefore, for DOPA$_2$–pCBMA$_2$ coated silica to be effective in cancer diagnostics, the functionalized surface must be able to detect similar biomarker concentrations. In this work, the limit of detection of ALCAM directly from human serum was found to be ~64 ng/mL, which is of similar magnitude to the other two platforms. The differences likely arise from the different surface densities and total amount of non-fouling and functionalizable CB groups.

4. Conclusions

A novel polymer conjugate containing ultra-low fouling and functionalizable zwitterionic pCB polymers and two DOPA moieties (for increased polymer surface coverage) was successfully grafted to SiO_2 coated SPR chips. SiO_2 is being increasingly incorporated into the design of novel NEMS devices which emphasize the importance of developing new surface chemistries for this material. Subsequent antibody functionalization enabled the detection of a target analyte directly from undiluted human serum with high sensitivity and specificity using a SPR sensor.

The attachment of the polymer conjugate was tested under a range of pH values, temperatures, and ionic strengths. Using the optimal conditions, a straightforward and convenient "graft to" method in room temperature Tris pH 8.5 buffer enabled a strong and mostly permanent surface coating to form on the silica surface. Subsequent biofouling experiments yielded undetectable adsorption to single protein solutions and low fouling to 100% human plasma and serum. The sensitivity of the DOPA$_2$–pCBMA$_2$ surface for the label-free detection of specific analytes directly from undiluted human serum was tested using the cancer biomarker ALCAM. Under optimized conditions, the detection limit was found to be ~64 ng/mL. The successful demonstration of the *in situ* attachment of DOPA$_2$–pCBMA$_2$ to SiO_2 coated SPR chips and subsequent detection abilities illustrate the potential this approach holds for the development of nano-scale sensors for medical diagnostics. Furthermore, the unique ultra-low fouling and functionalizable properties make this zwitterionic polymer promising for applications in implantable medical devices and *in vivo* diagnostics.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bios.2010.03.012.

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